EXPLORING AND SCRUTINIZING SUSTAINABLE ALTERNATIVE METHODS TO ENHANCE THE MEDICINAL POTENTIAL OF *GANODERMA SPECIES*

Research Protocol for Registration for Ph.D. Program

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CERTIFICATE

This is to certify that this Research proposal entitled "**EXPLORING AND SCRUTINIZING SUSTAINABLE ALTERNATIVE METHODS TO ENHANCE THE MEDICINAL POTENTIAL OF *GANODERMA SPECIES***" by Ms. Shweta Sharma, submitted in fulfilment of the requirement in Ph.D. program in Department of Botany under School of Sciences of JECRC University, Jaipur, Rajasthan, during the academic year 2025, is a Bonafide record of work carried out under our guidance and supervision.

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ANNEXURE-A

Section 1: Scholar's Basic Information

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INTRODUCTION:

The well-being of humans has been widely affected by mushrooms. Mushroom has manifolds, and it is one of the most heterogeneous groups of organisms on earth. Mushrooms are non-photosynthetic living bodies that develop above the earth on humus-rich soil and agro-residues as compost (Kumar *et al.,* 2023).

Mushrooms have a significant history from ancient times. Evidence shows that mushroom farming began in China (Zhang *et al.*, 2014). In France and other European countries, mushroom cultivation gained popularity in the 17th century. Since caves offered the ideal conditions for mushroom cultivation, French gardeners were the first to develop the growing technique.

In the early 1900s, mushroom farming gained popularity in the US. In 1896, W. Robinson founded the first commercial mushroom farm in the United States in Pennsylvania. However, it was not until the 1920s that mushroom farming took off in the United States, thanks to the knowledge and skills that European immigrants brought with them. Up until the middle of the 19th century, this technique was the norm for growing mushrooms and was later adopted in other parts of Europe.

Every year, millions of tonnes of mushrooms are produced around the globe, making mushroom farming a significant industry today. While compost and climate-controlled environments are still the primary methods for growing most mushrooms, innovative techniques are emerging that could revolutionize the market once again. There are different ways for sustainable mushroom cultivation, such as using food waste and agro-residues. Nowadays, Researchers use robotics and artificial intelligence to enhance mushroom properties and production.

Mushroom farming is still a vibrant and changing industry despite its long history. Asia provides a suitable environment (humidity and temperature, humus-rich loamy soil, under the forest, and Meadows) for mushroom cultivation. Solan is the first district of Himachal Pradesh in India where mushroom cultivation started in the mid-sixties. The most suitable areas in diverse India are the Western Ghats and the hills of northeast India. Nowadays, many states of

India are commercially producing mushrooms due to their significant economic value. Major mushroom-producing states are Himachal Pradesh, Punjab, Haryana, Uttar Pradesh, Madhya Pradesh, Tamil Nadu, Karnataka, and Andhra Pradesh. Sonipat, Panipat, and Gurgaon are three districts in Haryana that produce major mushrooms (Gupta *et al*, 2022).

Scientists estimate that there are nearly 14,000 species of mushrooms. Out of these, only about 200 are cultivated across the world, around 60 are considered to have real economic importance, and just 10 are grown on a large industrial scale. (Gupta *et al*, 2016).

Many species of mushrooms are used for their medicinal and nutritional aspects due to its significant properties, like blood-lipid-lowering effects, anti-cancer, anti-diabetic and anti-microbial effects. (Ambhore *et al,* 2024).

India is mainly an agricultural country, and its cultivated area is about 4.37%. Yearly, 620 million tons of agro-waste are produced by agricultural fields. This agro-waste can be recycled by mushroom cultivation (Raman *et al*, 2018). It will also be helpful to fill the nutritional gap for a large population of India. (Nadeeshani *et al*, 2023).

In India, the following species of mushrooms are commonly grown (Jain *et al*, 2024)

- Button Mushroom (*Agaricus bisporus*) - Maharashtra, Gujarat, Karnataka
- Oyster Mushroom (*Pleurotus species*) - Tamil Nadu, Kerala & West Bengal
- Shiitake Mushroom (*Lentinula edodes*) - Uttarakhand & Jammu-Kashmir
- Milky Mushroom (*Calocybe indica*) - Tamil Nadu, Andhra Pradesh & Karnataka
- Paddy Straw Mushroom (*Volvariella volvacea*) - West Bengal, Odisha, Assam
- Chanterelle Mushroom (*Cantharellus cibarius*) - Jammu-Kashmir, Uttarakhand & Himachal Pradesh
- Morel Mushroom (*Morchella species*) - Jammu-Kashmir, Uttarakhand & Himachal Pradesh
- Enoki Mushroom (*Flammulinavelutipes*) - Maharashtra, Gujarat, Karnataka
- Lion’s Mane Mushroom (*Hericium erinaceus*) - Tamil Nadu, Kerala & Karnataka
- Button-Shiitake Mushroom
- *Ganoderma* (*Ganoderma lucidum*) - Himachal Pradesh, Uttarakhand, Rajasthan

Rajasthan, which has a diverse range of climates and regions, makes it suitable for growing various types of mushrooms. The Aravalli Hills, Udaipur, Jaipur, and Jodhpur regions of Rajasthan are areas that are good for growing mushrooms (Jaiswal *et al*., 2024), (Chouhan & Kaur, 2024). Certain species are found in Rajasthan, especially in the densely forested and mountainous regions.

However, commercial mushroom cultivation is still not common in Rajasthan. It is beneficial for local employment because of its important qualities, and a standardised, environmentally friendly, sustainable alternative substrate composition is required for mushroom cultivation in various climates. (Royse *et, al*., 2017), (Kaushik *et al*, 2025).

Studies on *Ganoderma* species in Rajasthan suggest that the region has plenty of scope for both growing and researching this mushroom. The climate and local conditions make Rajasthan a promising place for its cultivation, (Jatav, 2007). The following reasons also support its cultivation-

- Raw material availability: A lot of agricultural waste is used as a substrate for the cultivation of *Ganoderma.* (Singh *et al.,* 2017).
- Government support: The government of Rajasthan provides a number of agricultural support programs, such as the Greenhouse program, Krishi Yantra program, and Rashtriya Krishi VikasYojana, which may help *Ganoderma* cultivation.
- Diverse climate: Rajasthan's varied climate can be used to grow *Ganoderma* in particular areas, greenhouses or another controlled environment.

Kingdom: Fungi

Phylum: Basidiomycota

Class: Agaricomycetes

Order: Polyporales

Family: Ganodermaceae

Genus: *Ganoderma*

https://www.researchgate.net/figure/Ganoderma-lucidum-Reishi-orLingzhi_fig1_347516960

Ganoderma mushrooms are also known as the "Mushroom of life enhancer and God of herbs," because they have many nutritional and medicinal benefits (Ihayere *et al.,* 2022; Cor *et al*., 2018). It has been historically used to treat a various disease in traditional Chinese medicine. Many researchers across the globe have tried *Ganoderma* cultivation on a variety of substrates. Its cultivation technique and biological activities change depending on the substrates and supplements used. (Jeewanthi *et al*, 2017).

Ganoderma lucidum, a wild mushroom species, has been historically used for medicinal purposes (Ekiz *et al,* 2023). The phytochemical analysis of *Ganoderma species* revealed the presence of various bioactive compounds (Marwein *et al*, 2025).

Because of this, researchers use alternative substrate compositions that can enhance the medicinal potential of *Ganoderma* for various drug formulations. Among these mushroom species, *Ganoderma* has significant medicinal and nutritional properties. The most valid total known *Ganoderma* species are 191, accepted world-wide (Zhou *et al*, 2024). Species such as *G. lucidum, G.applanatum, G.sinense, and G. amboinense* are commonly found in India and in many other parts of the world.In Rajasthan *G.lucidium,G.praelongum*, *G. capense, G.ausrale, G.applanatumare* commonly grown species. (Atri, 2024). *Ganoderma* cultivation is beneficial for local employment due to its environment friendly properties.

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Ganoderma cultivation is commercially beneficial these days due to:

- High market value
- Strong global demand
- Low input costs
- Medicinal properties
- Diverse applications as dietary supplements and pharmaceuticals.

Review of literature

In the present era, it is required to develop a sustainable approach in the agricultural sector by which maximum use of resources, minimum waste and carbon emission, and recycling can be possible, and mushroom farming is one of the ways to achieve these objectives for sustainable development. Different types of substrates are used for mushroom cultivation. Research shows that substrate composition leads to different mushroom production efficiencies; substrate formulation can affect mushroom productivity and its nutritional and medicinal properties. (Salichanh *et al*, 2025). Generally, compost and non-composted substrates can be used in commercial mushroom farming. (Kong *et al*, 2022).

Various agricultural materials can be used in mushroom cultivation as a mixture, such as sugarcane bagasse, corn cobs, soya stalk, sunflower stalks, banana leaves, cottonseed hull, and many agricultural residues. Good substrate increases the content and variation of metabolites, such as phenolics, in mushrooms (Mkhize *et al*., 2022). Different kind of supplements with substrate is used to improve medicinal value of mushroom (Nsoh *et, al,* 2022).

Mushroom cultivators and researchers have varying success with alternatives to substrates like wheat straw, sawdust, corn, cobs, and sugarcane straw (Hau Kongo *et al.,* 2021). However, there is still a gap in understanding the nutritional requirements of mushrooms during cultivation. Mushroom cultivators are encouraged to practice with locally available substrates due to the feasibility and economic point of view. (Geetha *et al.*, 2021). *Acacia* species and *Terminalia* have been used for mushroom cultivation. According to different regions, various possible local substrates must be found through study and research to find the best substrate and better ideas for mushroom cultivation.

Mushroom growing substrates should be formulated to enhance the nutritional and medicinal

properties of specific mushrooms and can be used for various purposes (Mkhize *et al*, 2022; Luo *et al*, 2024), While a lot of research exists on mushroom cultivation substrates, research on optimal substrate composition for various mushroom species in different climatic conditions is still limited. Research on tailoring substrates to achieve specific nutritional profiles (e.g., higher protein, specific vitamins/minerals) is lacking. (Marwein *et al, 2025).*

Research on the long-term sustainability, cost-effectiveness of different substrate compositions, and their impact on production cost are also needed to promote sustainable and economically viable mushroom cultivation practices. Notably, the characteristics of the growing substrates affect the presence of specific bioactive compounds in the mushroom. However, Nsoh *et al.* (2022), reported in *Pleurotus ostreatus* (oyster mushroom) that supplementing oyster mushroom substrate with *Rauwolfia vomitoria* can add anti-cancer properties to the oyster mushroom. The phytochemical analysis of *Ganoderma* revealed the presence of various bioactive compounds (Huyen *et al.,* 2025).

So, it is required to research different alternative substrate compositions that can enhance the medicinal potential of *Ganoderma* in different drug formulations.

Research Gap:

Despite advancements in substrate selection and fertilizer optimization (Shah *et al.,* 2018; Trung *et al*., 2024), limited research exists on the long-term sustainability of these practices. The circular economy approach, especially the reuse of spent mushroom substrate (SMS), is increasing but requires further research to ensure consistent quality and yield (Kousar *et al.,* 2024)

The microbial ecology found in the compost and casing layers during mushroom cultivation has not been studied as much as it should be (McGee, 2017). Gaining a better knowledge of these microbial communities enhances our cultivation techniques and helps to prevent issues related to spore production and post-harvest handling, especially in developing countries. As much research has already been done on preservation techniques and post-harvest processing methods there is a need for more research on maintaining the nutritional content and sensory properties of mushrooms during these processes. Research into *Ganoderma* has shown that using agro-residues to produce the medicinal mushroom *G. lucidum* commercially could

substitute chemical drugs in the future. However, more research is required for effective supplementation. (Luo *et al*, 2024)

In conclusion, addressing these research gaps could lead to more efficient, sustainable, and technologically advanced mushroom cultivation practices. Therefore, this study aims to understand and identify eco-friendly, sustainable alternative substrate compositions for *Ganoderma* mushroom species cultivation, and to investigate the effect of medicinal plant supplementation with the substrate.

Objectives:

- To **screen** agro-residues of Rajasthan for substrate preparation of *Ganoderma* species.
- To **analyse** medicinal plants as supplements in the substrate composition for *Ganoderma* cultivation.
- To **evaluate** the effect on the medicinal properties/bioactive compounds of *Ganoderma* species by using the medicinal plant as a substrate supplement.
- To **standardize** the method/composition of substrate for enhancing the medicinal properties of *Ganoderma* species.

Materials and Methods:

Setting & Sampling:

The sample spawn of *Ganoderma* species will be collected from DMR, Solan (HP). To obtain the pure culture and multiply the strain, the PDA (potato-dextrose agar) method will be used (Roy *et al.,* 2015). Cultivation technology provided by the Directorate of Mushroom Research, Solan, HP will be adopted in this study.

Substrate Preparation:

Sawdust (suitable, locally available *Acacia* species */Azadirachta* species/*Prosopis* species) will be mixed with bajra cobs, sugarcane bagasse in equal quantities to prepare a mixture with 20% wheat bran. Calcium Carbonate and Calcium Sulphate will be added at 2% to achieve a pH of 5.5; then the entire content will be adjusted to retain 65% moisture content in the substrate composition. This substrate will be filled into plastic bags (polypropylene bags, 18x25 cm),

and three replications will be made for each substrate. Tags will be placed on bags for clear identification and differentiation. The substrate bags will be tied and sterilized in an autoclave for 2 hours at 15 lbs. The packets will be transferred into a clean aseptic chamber and allowed to cool for 24 hours.

Substrate Preparation with Medicinal Plants:

Equal parts of some selected medicinal plants will be combined with the substrate. To ensure homogeneity, a pre-determined ratio of each will be added to the substrate and thoroughly mixed (Nsoh *et al*., 2022). The experiment will use different parts, like roots and leaves or the whole plant, of three selected medicinal plants, which are rich in specific bioactive compounds. They will be chopped, dried and ground to make powder. The grounded powder will be thoroughly mixed with the substrate, which will later be filled into the substrate bags. The pH of the substrate will be 4.5 to 6.5 for optimal growth. These bags will be sterilized for 4 hours in the labs. After 10-12 hrs, the bags will be removed from the lab and allowed to cool at room temperature. Using a planting spoon, we mix the spawn into the bags. The bags will be moved to the dark room to enhance colonization of the mycelia network. After around 20 to 22 days, the mycelium will be fully colonized. (Jeewanthi *et al*., 2017).

The temperature (25-30°C), relative humidity (80-85%), and proper ventilation will be maintained according to the requirements of *Ganoderma* for cultivation. (Nguyen *et al*, 2023).

Experimental data of different parameters will be collected during the growing process of *Ganoderma* fructifications such as mycelia growth period, primordial initiation and fruiting body initiation and maturation period required. After harvesting the fruiting body, phytochemical analysis will be done.

Phytochemical Analysis:

Ganoderma has various bioactive compounds like Triterpenoids, Phenolics, Glycosides, Alkaloids, Flavonoids, Saponins, and Polysaccharides& Proteins. (Boh *et al*, 2007). The major bioactive compounds are Triterpenoids, Polysaccharides and Proteins which are responsible for inducing specific medicinal properties in *Ganoderma* thallus for anti-cancer, antimicrobial, antioxidant, hepatoprotective, cardiovascular, and anti-inflammatory qualities. (Rijia *et al*, 2024).

The aim of the study is to quantitatively increase the percentage of major components especially Triterpenoids (lanostane-type triterpenoids -Ganoderic acid), Polysaccharides and proteins in the *Ganoderma* thallus by the addition of dried powdered different parts of medicinal plant in the basic substrate combination prepared for *Ganoderma* culture.

After regular time interval of the experiment setup the quantitative observation of phytochemical compounds will be performed as according to the objective of the research, using available standard testing methods i.e. both qualitative and quantitative. The Methods which will be applied during phytochemical analysis will be as follows-

Sample Preparation: After the harvesting of the fruiting body of *Ganoderma*, it will be chopped into pieces, and by drying and grinding, it will be converted in to a powder, and then Soxhlet extraction will be adopted to prepare the mushroom extracts using different solvents such as water, methanol and ethanol for phytochemical analysis (Hossain *et al.,* 2025), (Sharma *et al,* 2015).

Phytochemical Test: After extraction, the concentrated residues will be used to detect the secondary metabolites of *Ganoderma,* such as triterpenoids and polysaccharides.

- **Estimation of polysaccharides –**

  The carbohydrate content of dried *Ganoderma* is measured using a standard procedure (AOAC Association of Official Analytical Chemists; AOAC: Washington, DC, USA, 2012).

  - Quantitative polysaccharide analysis will be done according to AOAC guidelines and follow the basic two steps: first, acid hydrolysis in controlled conditions, which is followed by glucose analysis by GOPOD (glucose oxidase & peroxidase) or HPLC, and then enzymatic hydrolysis will be used to separate alpha-glucans to estimate beta-glucans. (Mc Cleary *et al*., 2016).

  - HPLC – First prepare the sample by filtration and centrifugation, then dilute it in the mobile phase, and then inject it into the HPLC system.

Deliver solvent at constant flow and through the HPLC column to separate compounds. With the help of UV detectors, it detects and quantifies eluted compounds. Use chromatogram output (peak retention time- identification and peak area for quantification) and then result interpretation by comparing with standards and calculating concentration.

- **Estimation of triterpenoids –**

  - Quantitative analysis of triterpenoids will be carried out with the help of HPLC-DAD (Huang *et al,* 2010), a validated RP-HPLC method (Chen *et al*., 2008) or LC-MS (Liquid Chromatography–Mass Spectrometry) or Colorimetric Assay/Screening by Vanillin-Perchloric Acid / Vanillin Sulphuric acid method. (Guo *et al.*, 2024). These methods will be used according to the needs and specifications of the experiment.

- **Estimation of protein –**
  - Protein analysis will be conducted using the Bradford Method.
  - Bradford Method –First, we will prepare a series of protein standards with known concentrations, then a sample will be quantified, and then Bradford reagent will be added to the sample and standards. After mixing well and incubating at room temperature for 5-10 minutes, we will measure the absorbance at 595 nm with a spectrophotometer. After that, we will create a standard curve and determine the sample concentration. (Bradford, M.M.,1976).

Validation:

AOAC (Association of Official Analytical Chemists) offers Guidelines for method validation, which includes study on specificity, linearity, accuracy, precision, and robustness. It recommends the validating analytical methods which are based on the following parameters-

1. Specificity – Analyse specificity of desirable bioactive compound in the presence of other components. It includes matrix interference, impurities, and degradation products.
2. Accuracy – Proximity of measured value to true or reference value. It uses Horwitz ratio (Hor Rat) to compare observed precision with predicted value.
3. Precision – Repeatability of the same/different analyst, same lab/ different lab, same/different equipment in a specific time frame. (It should be according to RSD-Relative Standard Deviation, which is ≤ 5-10 % on analytical level). This includes the use of statistical tools for precision estimates, like ANOVA
4. Robustness – Analyse the ability to remain unaffected with small changes in experimental conditions.
5. Linearity & range – Regression analysis can be used for analyse the linearity(a method to produce results proportional to the analyte concentration within a defined range)

The above guidelines will be followed during the phytochemical analysis process.

Scope and Significance:

Mushroom farming is exploding in India. The demand for edible mushrooms is soaring, and it's a perfect fit for farmers as it requires little investment, easy to manage, and has a short life cycle. As a result, the profitability from mushroom farming is potentially high. Previous research shows that differences in substrate, supplement type, and composition can influence the bioactive compounds found within mushrooms, which also affects the medicinal properties. Medicinal plants can be used as a supplement in substrate for mushroom growing so as to improve yield and medicinal potential. *Ganoderma* farming presents a lucrative opportunity to enhance farmers' income, with dried fruiting bodies commanding prices between ₹4,000 and ₹5,000 per kilogram.

Farmers are finding *Ganoderma* to be a great crop. The mushroom fetches a high price on the global market, and it doesn't take much money or resources to grow it. Government support, research initiatives, and growing consumer preference for herbal products further enhance its appeal.

If a phytochemical compound present in medicinal plants enhances the medicinal potential of

Ganoderma, then this research will:

- Encourage mushroom growers to grow and supplement their *Ganoderma* substrate with medicinal plants to improve and increase its medicinal as well as production value.
- Be helpful in identifying new locally available Agri-waste resources of Rajasthan that can be utilized in *Ganoderma* cultivation.
- Aim to find an alternative, sustainable, and standard method for *Ganoderma* cultivation.
- The outcomes of the research may provide insight for further research on medicinal mushrooms.

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